METABOLISM AND NUTRITION

*Bt*176 Corn in Poultry Nutrition: Physiological Characteristics and Fate of Recombinant Plant DNA in Chickens

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ABSTRACT A genetically modified *Bt*176 corn hybrid, which contains an insecticidal protein against the European corn borer, and its conventional, nonmodified counterpart were evaluated in 4 separate trials to verify substantial equivalence in feeding value and animal performance. Thirty-six individually kept laying hens and 3 replicates of 94 broiler chickens each, assigned to 12 cages, were fed 2 different hen and broiler diets containing either 60% conventional or 60% *Bt*176 corn. The nutrient compositions of the 2 corn hybrids and the 2 corn diets revealed no major differences. Furthermore, metabolism and performance data revealed no significant differences between the birds that received the conventional, nonmodified corn, and those that received the modified corn diets. The detection of the genetic modification, by PCR, in feed obtained from insect-resistant *Bt* corn, in tissues and products from animals fed *Bt* corn is described. In all evaluated chicken tissues of muscle, liver, and spleen, the corn-chloroplast *ivr* gene fragment was amplified. It can be deduced from these findings and from other studies that the transfer of DNA fragments into the body is a normal process that takes place constantly. Nevertheless, no recombinant plant DNA fragments such as recombinant *bla* or *cry*1A(b) fragments could be found. *Bt*-gene specific constructs from the *Bt* corn were not detected in any of the poultry samples, neither in organs, meat, nor eggs.

(*Key words*: transgenic feed, genetically modified, *Bt*176 corn, fate of DNA, substantial equivalence)

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INTRODUCTION

Genetically modified (GM) plants are those in which one or more new DNA constructs originating from foreign organisms have been integrated. The genetic modifications of plants are mainly aimed at herbicide tolerance, insect resistance, increased product quality (e.g., reduction of the content of undesirable antinutritive substances and increase of valuable substances like vitamins or fatty acids), and improvement of agronomical properties (e.g., low consumption of water and nutrients or drought tolerance) (reviewed in www.bio.org). With conventional plant breeding, most of these goals may be attained in the long term, but with genetic engineering, this is possible in the short term.

The products of crop biotechnology meet rejection in the public, especially in Europe (Tait, 2000; Atherton, 2002; McGarry Wolf and Domegan, 2002). These products often initiate emotional discussions. One concern is the potential translocation of transgenes into animal feed products. Other questions address antibiotic resistance genes as unnecessary cloning leavings, the potential allergenicity of the newly produced proteins, or the substantial equivalence of the transgenic and the original plants.

The *Bt*176 corn was introduced to the market in 1996. It is resistant against the European corn borer, which causes serious damage each year in different parts of the world (www.extension.umn.edu/distribution/cropsystems/DC7055.html). Many of the GM corn plants, including *Bt*176 corn, contain the gene for *cry*1A(b) protein originating from *Bacillus thuringiensis* (*Bt*). This protein is an endotoxin that is highly specific for certain Lepidopterans such as the European corn borer. This specificity is due to a unique binding site in the midgut epithelium membrane of the insects, where the protein inserts and causes damage in the wall. In other organisms, the *Bt* protein is digested similarly to other proteins and has therefore no deleterious effects. Furthermore, *Bt*176 corn is genetically modified to express the *bar* gene cloned from the soil bacterium *Streptomyces hygroscopicus*, which encodes a phosphinothricin-N-acetyltransferase enzyme. This enzyme is useful as a selection marker. Finally, the *Bt*176 corn contains a beta-lactamase encoding a *bla* gene used as a selectable marker for screening bacterial colonies for the presence of the plasmid vector. The *bla* gene is not expressed in plant cells (Fearing et al., 1997).

One objective of animal nutrition studies using plants modified with agronomic traits is to assess whether the

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modified plants are nutritionally equivalent to their conventional counterparts. An increasing number of studies have reported the substantial equivalence of GM crops and their conventional, nonmodified counterparts in terms of nutrient composition and animal performance (Aulrich et al., 1998, 2001a, 2001b; Mireles et al., 2000; Clark and Ipharraguerre, 2001; Flachowsky and Aulrich, 2001; Chrenková et al., 2002).

Food-ingested foreign DNA is not completely degraded in the gastrointestinal tract. Research on the fate of foreign DNA in the mammalian organism showed that PCR products specific for foreign DNA could be detected therein. It was concluded that DNA fragments from the gastrointestinal tract could reach the bloodstream and be transported through the epithelium of the gut and the cells of the Peyer's patches to cells of spleen and liver. Such DNA fragments are probably retained for a short while and then digested (Schubbert et al., 1994; 1997; 1998). It seems that the intestinal tract is not an absolute barrier against the uptake of macromolecules or even of microorganisms, but the mechanism of foreign DNA uptake by the intestinal wall epithelia is unknown. In addition, not much is known about the degradation and the integration of the DNA. There is some evidence that fragments of foreign DNA are not digested in the gut and might enter the organism or become incorporated into cells lining the gut wall (Doerfler, 2000; Tony et al., 2003). Previous studies with *Bt* corn showed that native plant DNA fragments of different lengths could be associated with organs and muscles in beef, pork, and poultry (Einspanier et al., 2001; Reuter et al., 2002).

It is assumed that free nucleic acids are absorbed faster than nucleoproteins (Doerfler, 2000). Schubbert et al. (1997) proved by their experiments with mice that foreign DNA could be integrated into the genome of cells, mainly leukocytes. Probably this way of absorption is a resorption of DNA bound to proteins. Such complexes arrive at the immune competent cells through specialized gut cells. Therefore, the use of protease was meant to aid the degradation of these proteins and as a consequence to destroy such DNA-protein complexes faster.

The aim of our studies was to verify whether the nutrient compositions of 2 varieties of corn were equivalent. Furthermore, it was of interest to study whether there were differences in performance and metabolism of laying hens and broilers fed modified corn or the conventional nonmodified control corn, and to study a possible influence of the modification in the *Bt* corn on the quality of eggs and meat in terms of nutrient composition. In addition, we wanted to study the means of inactivation of nucleic acids, transgenic or nontransgenic DNA, in the digestive tract of broilers as well as to verify a possible transfer to chicken tissues like spleen, liver, blood, muscles, and eggs. To increase the degree of inactivation of the nucleic acids in the digestive tract, the enzyme protease was added to certain experimental diets.

TABLE 1. Nutrient and energy content of control (Prelude) and *Bt*176 corn

Constituent	Conventional corn	*Bt*176 corn
Dry matter (DM; g/kg)	875	876
In dry matter (g/kg of DM)		
Crude ash	13.9	13.6
Crude protein	89.7	88.2
ADF[1]	40.4	32.9
NDF[2]	140.7	135.9
Fat	35.7	35.2
Gross energy (MJ/kg)	16.78	16.69
Amino acids (g/kg)		
Lysine	2.40	2.50
Methionine	2.40	2.40
Cysteine	2.30	2.15
Tryptophan	0.66	0.64
Alanine	7.80	7.90
Arginine	3.90	3.95
Aspartic acid	6.50	6.50
Glutamic acid	21.00	20.20
Glycine	3.30	3.30
Histidine	2.70	2.80
Isoleucine	3.40	3.20
Leucine	13.70	13.80
Phenylalanine	5.10	5.10
Proline	9.50	9.00
Serine	4.90	4.90
Threonine	3.70	3.65
Tyrosine	4.10	4.10
Valine	4.90	4.90
Mycotoxin analyses		
Desoxynivalenol (μg/kg)	<200	<200
Zearalenone (μg/kg)	<50	<50

[1]ADF = acid detergent fiber.

[2]NDF = neutral detergent fiber.

MATERIALS AND METHODS

Experimental Design, Birds, and Diets

Two isogenic varieties of corn, conventional (Prelude) and the transgenic *Bt*176 corn, were grown, harvested, and stored under the same environmental conditions (Syngenta facilities, Germany). Grains harvested from *Bt*176 and from nonmodified corn were strictly separated, avoiding any possibility of commingling or contamination. Nutrient and energy content of both corn varieties are compiled in Table 1.

Details of the diet preparation and of the design of the broiler experiment have been reported previously (Aeschbacher et al., 2002). Three experiments each with 94 male broiler chickens at 1 d old (Ross 208) were performed. The broilers were assigned to 4 treatment groups with 3 cages (0.92 × 0.54 × 0.83 m) per treatment, with an equal distribution of body weight. The diets were prepared with transgenic *Bt*176 corn or with conventional, nonmodified corn, supplemented with protease[2] (1 g/kg) or not (Table 2). Diets corresponded to the nutrient requirements of broilers (NRC, 1994).

In the feeding experiment with laying hens (Isabrown, 17 wk old at beginning of the experiment), 36 hens, in 2 groups of 18, were randomly assigned to 1 of the 2 treatments. They were kept in individual cages (0.82 × 0.67 × 0.79 m) from 17 to 43 wk of age under laboratory

[2]Allzyme Vegpro, Alltech, Lexington, KY.

TABLE 2. Composition and nutrient content of the diets for hens and broilers

Ingredient	Experimental Diets					
	Hen Control	Hen *Bt*176	Broiler Control	Broiler Control	Broiler *Bt*176	Broiler *Bt*176
Corn	60	60	60	60	60	60
Soybean meal	17.5	17.5	30	30	30	30
Dried grass	5	5	—	—	—	—
Limestone flour	4.5	4.5	—	—	—	—
Limestone grit	4.0	4.0	0.8	0.8	0.8	0.8
Potato protein	3.0	3.0	2.2	2.2	2.2	2.2
Soy oil	2.5	2.5	3.5	3.5	3.5	3.5
Salt	0.15	0.15	0.1	0.1	0.1	0.1
DL-Methionine	0.2	0.2	0.25	0.25	0.25	0.25
Lysine-HCl	—	—	0.15	0.15	0.15	0.15
DCP 38/40[1]	1.35	1.35	1.2	1.2	1.2	1.2
Sodium carbonate	0.3	0.3	0.3	0.3	0.3	0.3
Celite	1.0	1.0	1.0	1.0	1.0	1.0
Premix[2]	0.5	0.5	—	—	—	—
Premix[3]	—	—	0.5	0.5	0.5	0.5
Protease	—	—	—	0.1	—	0.1
Calculated values						
Dry matter (DM; g/kg)	888	888	880	880	880	880
CP (g/kg)	160	160	240	240	240	240
Metabolizable energy (MJ/kg)	11.65	11.65	13.08	13.08	13.08	13.08
Actual analysis						
DM (g/kg)	902	901	890	896	891	894
In 88% DM						
Crude ash (g/kg)	134.9	133.0	58.3	58.4	60.7	59.8
Crude protein (g/kg)	169.8	173.1	180.9	184.8	197.3	187.5
Acid detergent fiber (g/kg)	50.4	51.0	49.3	49.3	54.2	51.5
Neutral detergent fiber (g/kg)	120.4	122.6	131.3	140.0	136.5	140.3
Fat (g/kg)	45.9	45.2	61.7	60.3	61.4	62.7
Gross energy (MJ/kg)	15.26	15.33	17.08	17.12	17.09	16.98

[1]DCP = Dicalcium phosphate.

[2]Hen premix: UFA, Sursee, Switzerland. One kilogram of feed contains: vitamin A, 12,500 IU; vitamin D_3, 2,000 IU; vitamin E, 30 mg; vitamin K3, 3 mg; thiamin, 2 mg; riboflavin, 5 mg; pyridoxine, 4 mg; cobolamine, 0.02 mg; biotin, 0.05 mg; pantothenic acid, 10 mg; niacin, 40 mg; folic acid, 0.5 mg; choline, 300 mg; betaine, 300 mg; Cu, 5 mg; Fe, 20 mg; Zn, 50 mg; Mn, 80 mg; Co 0.2 mg; I, 1 mg; Se, 0.25 mg.

[3]Broiler premix: UFA, Sursee, Switzerland. One kilogram of feed contains: vitamin A, 12,500 IU; vitamin D_3, 2,000 IU; vitamin E, 30 mg; vitamin K, 1.9 mg; thiamin, 2 mg; riboflavin, 5 mg; pyridoxine, 3.95 mg; cobolamine, 0.02 mg; biotin, 0.21 mg; choline, 290 mg; pantothenic acid, 20 mg; niacin, 40.5 mg; folic acid, 1.57 mg; betaine, 300 mg; Cu, 5.5 mg; Fe, 19.5 mg; Zn, 85 mg; Mn, 82.5 mg; Co 0.21 mg; I, 0.31 mg; and Se, 0.25 mg.

[4]Protease: Alltech Inc. Nicholasville, KY.

conditions. The stable temperature was 22°C and the hens were provided light for 14 h/d. They were fed a standard diet containing 60% corn, either conventional or *Bt*176 corn, and other components as shown in Table 2. Celite 545 was added to all diets as an inert indicator to estimate the metabolizability of nutrients and energy. The nutrient composition conformed to the standard requirement for laying hens (NRC, 1994). The unpelleted feed and water were provided ad libitum.

Sampling Procedure

Broilers. The performance of the broilers was measured weekly. Total cage weight and total cage feed intake were monitored weekly. Total cage water consumption was recorded for 4 d each week. Feed efficiency was calculated for each pen by dividing total feed consumption by weight gain. Losses were recorded and the apparent cause of death determined. Collection of excreta samples from every cage in all experiments was conducted in the third and the fifth week of the experiment to determine dry matter content, energy, and nitrogen utilization. After 39 d, the broiler chickens were slaughtered in a commercial poultry abattoir. Carcass weight and weights of spleen, liver, and heart were recorded. The right breast muscle was resected, freeze-dried, and ground in a laboratory grinder[3] for nutrient composition analysis.

To collect digesta samples from the intestinal tract (crop, gizzard, small intestine, and cecum), as well as samples from meat and organs (spleen, liver) after 14, 28, and 38 d, one animal from each group was killed by decapitation and bleeding. In doing so, blood was collected in anticoagulant, centrifuged, and stored at –25°C until PCR analysis.

Laying Hens. Hen weight was recorded once a month and feed intake once a week. Number and weight of eggs were recorded daily. Eggs were collected during 1 wk each mo throughout the experiment (6 times). The col-

[3]Moulinette, Moulinex, Paris, France.

lected eggs of each hen were merged, freeze-dried, and ground in a laboratory grinder. Collection of excreta random samples from each hen was conducted 3 times on 3 d throughout the study to determine energy and nitrogen utilization and excreta dry matter. Blood samples were obtained by wing venipuncture and collected in anticoagulant. The samples were centrifuged and then stored (–25°C) until analysis.

Analytical Methods

Compositions of feeds and excreta were determined. During feed preparation and throughout every excreta collecting period, samples of feed were collected and milled through a 0.75-mm screen. The excreta samples were stored frozen and the samples of each hen merged at the completion of the collection period, dried in an oven at 60°C for 48 h and ground (0.5 mm) for proximate analyses. Contents of dry matter (DM), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF) in corn and feedstuffs were determined according to standard methods (Naumann and Bassler, 1997). Nitrogen content was determined with a C/N analyzer[4] by the Dumas method. Crude protein was calculated as 6.25 × N. The gross energy content was determined by an anisothermic bomb calorimeter.[5] Fat content in feed and muscle was determined gravimetrically as petroleum-ether extract. Total DM of excreta was computed by moisture loss during drying at 60°C for 48 h and drying at 104°C for 4 h. The proteins in the eggs were determined by the method of Kjeldahl (Lebet et al., 1994). The metabolizability of energy and the N-balance were determined by the indicator method, using 4 *N* HCl-insoluble ash as the indicator (Prabucki et al., 1975). An external laboratory analyzed mycotoxins.

Statistical Analysis

The experimental data were analyzed statistically using the Statgraphics Plus program for Windows[6] (1997). Means were compared using Bonferroni's test at a significance level of $P < 0.05$.

PCR Analysis

DNA Extraction. DNA was extracted from corn and feed samples, eggs, samples from the broilers' muscle, liver, and spleen, as well as digestive contents from the crop, gizzard, small intestine, and cecum, and from excreta and blood of hens and broilers. These different samples were taken to retrace the partial degradation of the DNA in the digestive tract.

Eggs were disinfected with 70% ethanol before being cracked. The egg content was homogenized in a Stomacher for 3 min. All tissue samples were excised from the middle of the organ with a sterile scalpel blade to minimize contamination from the surface. Freshly drawn blood was diluted twice with an anticoagulant and centrifuged at 6,000 rpm for 10 min. Samples were collected from the plasma (top) and from the pellet and resuspended in TritonX-100. The extraction of DNA from most samples was carried out according to 2 methods (below), whereas DNA from muscle and organs samples was extracted by method 2 alone. DNA was isolated from certified corn reference material by method 1.

Method 1: 100 mg of the sample was mixed in a plastic tube with 200 μL of sterile distilled water and 1 mL of buffer (55 m*M* cetyl-trimethyl-ammonium bromide, 1,400 m*M* NaCl, 100 m*M* Tris-HCl, and 20 mM EDTA; pH 8.0) and incubated at 65°C for 1 h. After adding 40 μL of proteinase K (20 mg/mL), the sample was incubated for another 3 h at 58°C, and then centrifuged at 20,000 × *g* for 10 min. The supernatant was transferred to another tube, mixed with 400 μL of chloroform, and centrifuged (20,000 × *g*; 10 min). This step was repeated twice. The resulting supernatant was mixed with an aliquot of isopropanol and centrifuged. The extracted DNA was purified according to the Wizard isolation protocol.[7]

Method 2: 300 mg of sample was mixed in a plastic tube with 860 μL of extraction buffer (10 m*M* Tris-HCl, 150 m*M* NaCl, 2 m*M* EDTA, 1% SDS; pH 8.0), 100 μL of 5 *M* guanidine hydrochloride, and 40 μL of proteinase K (20 mg/mL), incubated at 58°C for 4 to 15 h (overnight), and centrifuged at 14,500 × *g* for 10 min. The supernatant was purified using the Wizard Kit.[7]

PCR. All PCR reaction samples were performed on a 96-well cycler.[8] Specific primers[9] for the corn-specific invertase gene *ivr* (226 bp) (Ehlers et al., 1997), a poultry-specific DNA fragment (227 bp) (Matsunaga et al., 1999), and for the *Bt*176-specific foreign gene *bla* (479 bp) encoding an ampicillin-resistance determinant (Aeschbacher et al., 2002) were used. Primers were supplied in lyophilized form and stored at −20°C.

Control amplifications verified the lack of contaminating DNA after extraction and excluded the existence of inhibitors in the PCR solution. The PCR was performed according to the following conditions:

The first cycle was initiated by a denaturation step at 95°C (94°C for poultry-specific PCR) for 3 min. Each cycle consisted of denaturation at 95°C (94°C for poultry-specific PCR) for 30 s, annealing at hybridization temperature for 30 s, and extension at 72 °C for 3 min, or for 30 s with poultry-specific primers. Hybridization temperature was 64°C for *ivr*-specific PCR, 65°C for *Bt*176-specific PCR, and 60°C for poultry-specific PCR. After 35 cycles (42 cycles for *ivr*-specific PCR), the reaction continued for 10 min (7 min for *Bt*176-specific PCR) at 72°C, and was then cooled to 4°C or frozen until further use. The PCR products were fractionated by horizontal electrophoresis in 1.2 to 1.5% agarose gels and stained with ethidium bro-

[4]FP-2000, Leco Instruments GmbH, Cheshire, UK.
[5]IKA-Kalorimeter C 7000, IKA Werke, Staufen, Germany.
[6]Manugistics Inc., Rockville, MD.
[7]Promega, Madison, WI.
[8]Techne Genius Thermal Cycler, Jepson Bolton & Co. Ltd., Herts, UK.
[9]Microsynth, Balgach, Switzerland.

mide. A kilobase ladder, containing linear DNA fragments, served as size standard reference.

A single reaction mixture of 50 μL contained 10% optimized 10× PCR buffer,[10] 2.5 m*M* $MgCl_2$, 2 μg/mL BSA, and 0.2 m*M* dNTP (nucleotides). In addition, the mixture contained 0.5 μM of forward and 0.5 μM of reverse primer, and 1.0 U of Taq DNA polymerase (5.0 for *Bt*176-specific PCR). Primers for poultry-specific PCR were added at 0.5 μM (forward) and 1.0 μM (reverse) (Matsunaga et al., 1999). The DNA samples were added from 1 to 5 μL, depending on their concentrations.

RESULTS

Nutrient Content of Corn Varieties and Experimental Diets

In order to compare nutrient fractions of the 2 corn lines, DM, crude ash, CP, ADF, NDF, fat, and gross energy were analyzed. The analyzed nutrients of the *Bt* corn and the conventional, nonmodified corn showed no major differences (Table 1). In addition, comparison of the analytical results of both corn types with the composition data on corn from the literature revealed no differences. All results were close to published values or within the range of the standard deviation.

Amino acid analyses showed very similar amino acid patterns for samples of transgenic and conventional corn. No mycotoxins such as deoxynivalenol and zearalenone were detected in the corn samples.

To compare the composition and nutrient content of the control and *Bt*176 corn diets for hens and broilers, DM, crude ash, CP, ADF, NDF, fat, and gross energy were analyzed (Table 2). No differences in nutrients and energy content were detected between the corresponding diets, as expected given that the corn as main component in the diet did not differ in nutrient and energy content (Table 1). The actual data correspond well to those calculated in advance. The higher contents of CP and ADF in the *Bt*176 feed without protease must be attributed to an error in the production of this feed for one experiment.

The supplementation of the broiler feed with protease had no influence on the nutrient composition of the feed (Table 2). An influence of the protease can be excluded because this effect happened in one experiment and only in that particular feed.

Performance Data of Laying Hens and Broilers

In laying hens no performance parameters differed ($P > 0.05$) between the 2 experimental diets (Table 3). The *Bt* corn had no influence on the weight gain of the hens. The daily feed intake did not differ significantly between the 2 feeding groups and was 109.6 and 117.8 g/bird per d for the control and *Bt* group, respectively. The laying rate during the experiment was within the expected range and was 94.5% for the control group and 95.7% for the *Bt* group. Feed efficiency was not affected by the corn type; feed efficiency was 1.81 and 1.89 g of feed per g of egg in the control and *Bt* group, respectively.

TABLE 3. Effect of corn variety in the diet on performance parameters of hens (n = 18) during 6 mo[1]

Parameter	Control group	*Bt*176 group
Initial BW,[2] (g)	2,008 ± 101.87	1,931 ± 84.43
Final BW,[3] (g)	2,151 ± 194.27	2,019 ± 98.79
Laying rate LP1,[4] (%)	94.5 ± 6.3	97.6 ± 5.2
Laying rate LP2, (%)	89.3 ± 11.9	95.4 ± 12.5
Laying rate LP3, (%)	93.1 ± 9.06	97.6 ± 3.24
Laying rate LP4, (%)	95.0 ± 6.06	93.3 ± 6.92
Laying rate LP5, (%)	93.9 ± 6.53	89.7 ± 10.9
Laying rate LP6, (%)	91.2 ± 6.7	82.9 ± 16.1
Laying rate LP1-6, (%)	94.5 ± 4.7	95.7 ± 2.6
Daily feed intake (g/bird per d)	109.6 ± 5.0	117.8 ± 8.1
Feed efficiency (g of feed/g of egg)	1.81 ± 0.13	1.89 ± 0.12

[1]Results are mean ± SD.
[2]BW at 17 wk of age.
[3]BW at 43 wk of age.
[4]LP = laying period.

The results of the experiments in terms of BW, weight gain, daily feed intake, and feed conversion ratio (Table 4) were not significantly influenced by the variety of corn. The use of conventional corn led to a feed conversion ratio (d 1 to 38) of 1.632 g/g and a weight gain of 56.0 g/d, and that of the transgenic corn to 1.649 g/g and 55.4 g/d, respectively. Daily feed intake was 91.4 and 91.3 g for conventional and modified corn varieties, respectively. Water consumption (mL/g of feed) and slaughter weight per bird did not differ between the diets.

During the broiler experiments, no disturbances in behavior (ingestion, water intake, feather or leg picking), or animal health issues such as weight loss and leg disorders were observed. Two birds in the *Bt* group died during the experiments, due to ascites and sudden death syndrome, probably associated with the rapid weight gain and not related to the treatment. A mortality rate of 2% is commonly observed in such feeding studies.

Metabolism Data of Laying Hens and Broilers

In Table 5, the metabolism parameters of broilers and laying hens are displayed. Approximately 78% of the energy was digested by both hens and broilers. The AME content of the feed in the broilers amounted to 13.34 and 13.23 MJ/kg of DM in the conventional and *Bt* group, respectively. No difference in AME content of the feed in hens was detected (12.02 vs. 12.05 MJ/kg of DM). The nitrogen utilization coefficients and excreta DM content were not significantly influenced by the corn variety in either type of chicken. Nitrogen utilization in broilers accounted for 0.66 in the control group and 0.63 in the *Bt* group. In hens, nitrogen utilization was 0.48 and 0.44 in the control and modified group, respectively.

[10]Amersham, Bucks, UK.

TABLE 4. Effect of corn variety in the diet on performance parameters of broilers[1]

Analyses	Conventional corn	*Bt*176 corn
BW at d 1 (g)	42.5 ± 2.0	42.7 ± 1.5
BW at d 14 (g)	419.3 ± 16.2	422.0 ± 14.3
BW at d 28 (g)	1,386.7 ± 70.3	1,409.6 ± 55.1
BW at d 39 (g)	2,227.1 ± 114.5	2,207.1 ± 115.5
Daily BW gain, d 1 to 39 (g/d)	56.0 ± 2.4	55.4 ± 3.5
FCR,[2] d 1 to 38 (g/g)	1.632 ± 0.034	1.649 ± 0.058
Daily feed intake, d 1 to 38 (g)	91.4 ± 3.9	91.3 ± 4.2
Water consumption, d 8 to 38 (mL/g of feed)	1.86 ± 0.14	1.96 ± 0.17
Slaughter weight (g)	1,580.5 ±	1,571.0 ±

[1]Data from 3 experiments (6 cages per treatment each); mean value and standard deviation.
[2]FCR = feed conversion ratio (g of feed per g of weight gain).

Composition of Eggs and Meat

The nutrient composition of the product meat in terms of DM, CP, and fat was comparable (data shown in Aeschbacher et al., 2002). The results showed that there was no significant difference in composition between the 2 corn types. The same could be shown with the analysis of the eggs in terms of DM, protein, and amino acids (Table 6). Protein content was 219.74 g/kg of DM in the control group eggs and 225.17 g/kg of DM in the *Bt* group eggs. In addition, the comparison of the amino acid analyses revealed no significant differences between the eggs from the 2 feeding treatments.

Fate of Corn Chloroplast ad Bt Corn Specific DNA

Polymerase chain reaction studies were carried out to find DNA fragments from corn, either native fragments such as the corn chloroplast gene *ivr* or transgenic fragments from the *Bt* corn. For this purpose, corn-specific, poultry-specific, and *Bt*176-specific primer pairs were used in the PCR analysis. Figures 1 (hen data) and 2 (broiler data) show the results of the PCR analysis. Both corn varieties, the 2 kinds of feed, the digesta samples from crop, gizzard, small intestine, and cecum, the excreta samples as well as the samples from blood, muscles, eggs, and organs were used as PCR target material. A corn-specific fragment *ivr* (226 bp), a *Bt*176-specific fragment (479 bp), and a poultry-specific fragment (227 bp) were searched for. The number of broiler samples was 9 for each feed treatment; eggs, excreta, and blood samples from hens were randomly selected from within a feeding group.

Initial tests with conventional and transgenic corn samples served as control for detecting *Bt* corn or corn in general by the invertase gene *ivr*. All corn and feed DNA extracts resulted in a strong positive signal for the corn-specific fragment. In contrast, PCR reactions of those corn and feed extract samples with poultry-specific primers did not generate any PCR product. The PCR for the *Bt*176-specific fragment showed only strong positive signals for the GM corn and feed samples and no signals for the conventional corn samples. With all DNA extracts from the broiler samples, poultry-specific fragments were detected. Additional negative and positive controls verified (also with GM standards) that each PCR reaction was functioning properly.

Results of PCR of the poultry samples were positive regarding the poultry-specific analysis, with the excep-

TABLE 5. Influence of conventional and transgenic *Bt*176 corn in the diet on metabolizability of energy and nitrogen utilization of broilers and laying hens

Analysis	Conventional corn	*Bt*176 corn
Broilers[1]		
Metabolizability of energy	0.781 ± 0.013	0.775 ± 0.014
AME (MJ/kg of feed[2])	13.34	13.23
Nitrogen utilization	0.66 ± 0.063	0.63 ± 0.057
Excreta dry matter (g/kg)	297 ± 54	294 ± 46
Laying hens[3]		
Metabolizability of energy	0.788 ± 0.005	0.786 ± 0.004
AME (MJ/kg of feed)	12.02	12.05
Nitrogen utilization	0.48 ± 0.015	0.44 ± 0.010
Excreta dry matter (g/kg)	234 ± 20	239 ± 40

[1]3 × 2 × 6 samples per treatment.
[2]88 % dry matter.
[3]3 × 9 samples per treatment.

TABLE 6. Selected nutrient fractions and amino acids in eggs from laying hens fed diets containing conventional or *Bt*176 corn[1]

Analyses	Control corn eggs	*Bt*176 corn eggs
Dry matter	234.0	230.0
Protein	219.74	225.17
Amino acids		
Lysine	10.55	9.41
Methionine	4.54	4.10
Cysteine	3.35	2.91
Alanine	8.16	7.98
Arginine	7.85	8.50
Aspartic acid	12.14	13.85
Glutamic acid	15.19	17.06
Glycine	4.86	5.04
Histidine	3.67	3.27
Isoleucine	7.32	6.69
Leucine	12.34	11.22
Phenylalanine	7.81	6.87
Proline	5.47	5.11
Serine	11.80	10.86
Threonine	6.95	6.38
Tyrosine	6.07	5.19
Valine	9.27	8.74

[1]Average values (in g/kg) of freeze-dried egg mixtures of each hen of a treatment.

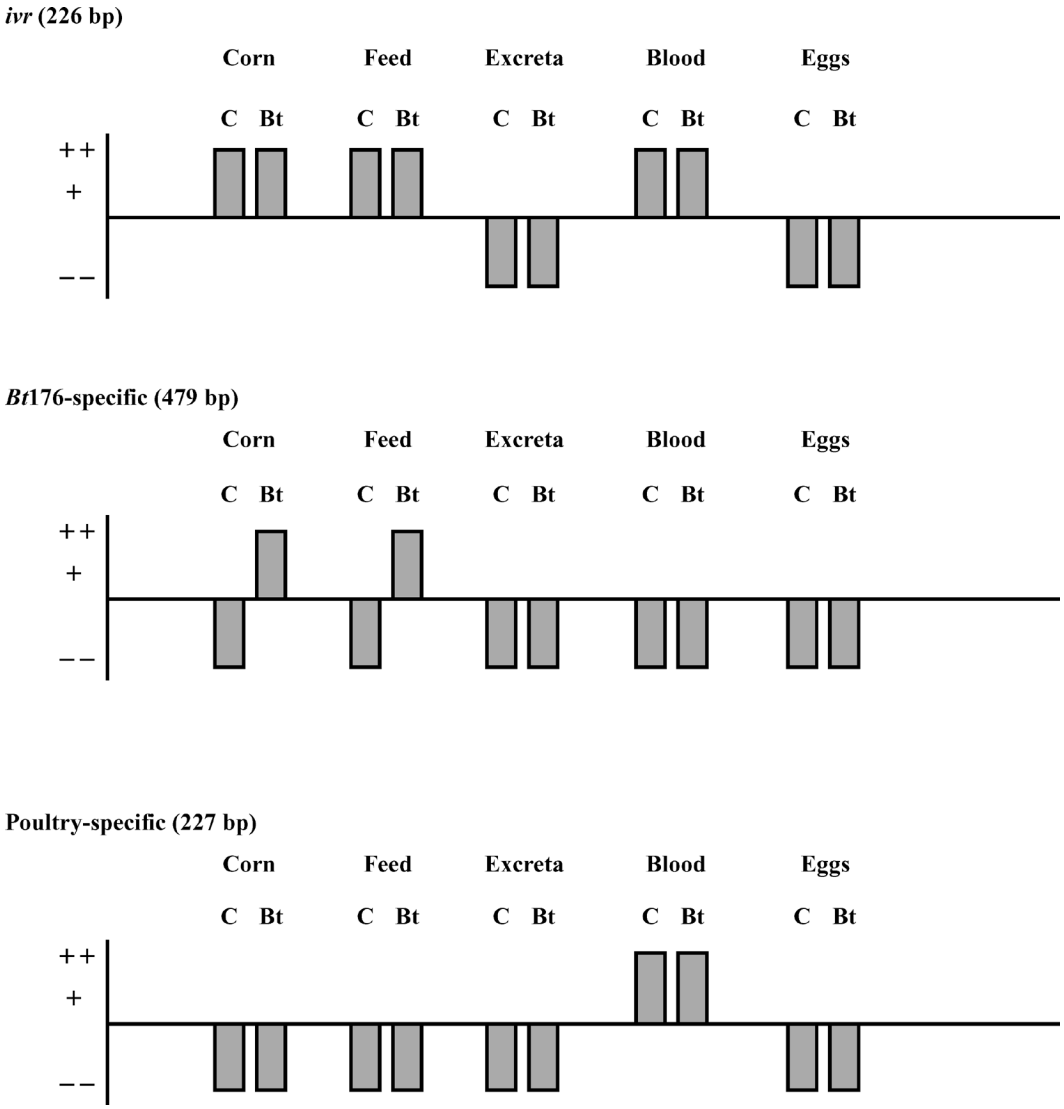

FIGURE 1. Polymerase chain reaction analysis of corn, hen feed, and hen samples. C = conventional, nonmodified corn; Bt = modified corn (*Bt*176);++ = intense signal; + = faint signal; – – = no detection.

tion of the eggs. The corn-specific gene *ivr* could be detected in corn, feed, and in digesta samples as far as the small intestine (only weak signals). In organs and blood, as well as in muscle samples (only weak signal), the *ivr* gene could be amplified. The *Bt*176-specific gene *bla* could only be detected as far as the crop. No transgenic DNA had been detected in meat, organs, blood, or eggs.

DISCUSSION

Nutrient Content of Corn and Experimental Diets

The *Bt* corn was modified to protect itself against a devastating pest. No modifications on the nutrient composition were made; therefore, the *Bt* corn should be equal to the original line. Our findings support the results of Brake and Vlachos (1998). They were the first to investigate *Bt*176 corn and the nontransgenic control and found nutrient contents to be similar.

In contrast to other studies, we used a high amount of corn in the diets (60%). No statistically significant differences were detected when the *Bt* feeds and their respective nonmodified controls were compared for their ingredients. Other comparative studies found substantial equivalence between a GM crop plant and its conventional counterpart, with no changes in its nutrient composition or in the feeds made with both corn varieties (e.g., Aulrich et al., 1998; Halle et al., 1999; Mireles et al., 2000, Böhme et al., 2001; Cromwell et al., 2002). Similar studies from Gaines et al. (2001) and Piva et al. (2001) with broilers fed GM corn (MON810) came to the same conclusions.

Performance of Laying Hens and Broilers

The present results suggest strongly that there are no deleterious effects on performance associated with the diets made from transgenic corn compared with diets made from nontransgenic corn. Our results confirm the findings from Mireles et al. (2000) and Gaines et al. (2001).

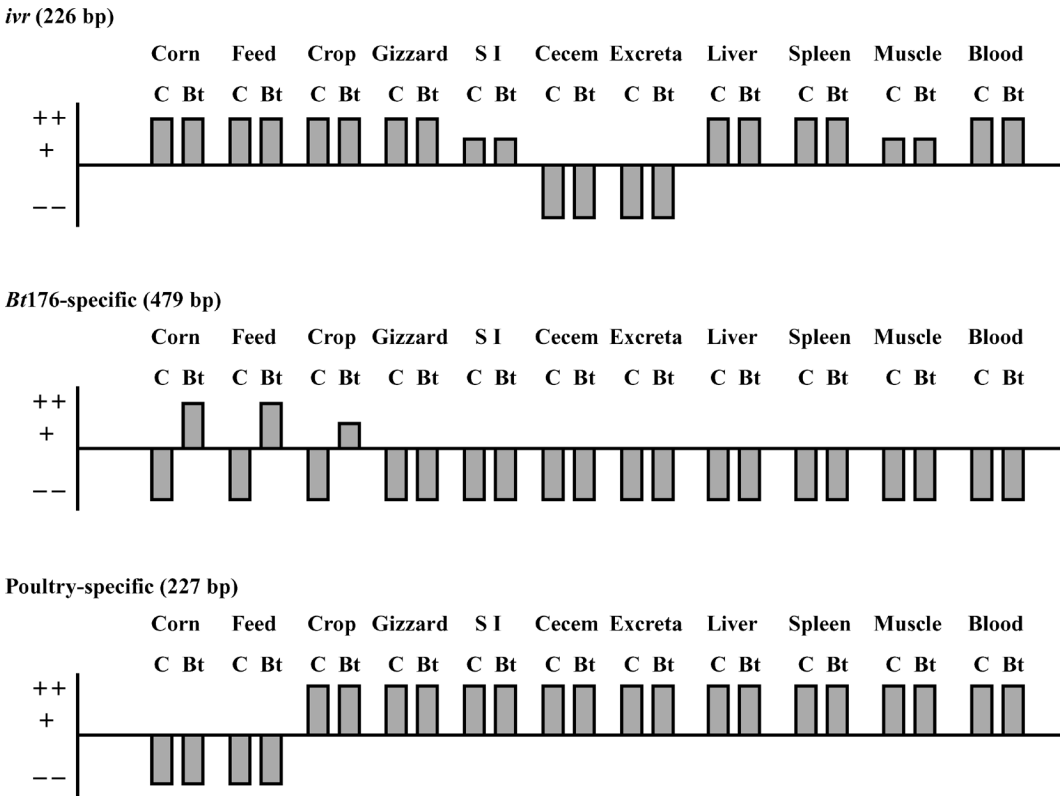

FIGURE 2. Polymerase chain reaction analysis of corn, broiler feed, and broiler samples. C = conventional, nonmodified corn; Bt = modified corn (*Bt*176);++ = intense signal; + = faint signal; – – = no detection; SI = small intestine.

Previous work has shown that in experiments with broilers or laying hens, neither feed intake, feed conversion ratio, nor weight gain were influenced by the genetic modification of the crop. In contrast to this, a study by Brake and Vlachos (1998) documented that broilers fed transgenic *Bt*176 corn exhibited a significantly better feed conversion ratio than those fed conventional corn. This effect could be explained by the occurrence of more mycotoxins (aflatoxins, deoxynivalenol, fumonisin) in the conventional corn, not protected against the European corn borer. In the corn used in this study mycotoxins were below detectable levels (Table 1).

Metabolism of Laying Hens and Broilers

Because of the substantial equivalence of the feed, no differences in metabolizability of energy or in nitrogen utilization were expected or observed. No influence of the corn type could be observed on energy and nitrogen utilization or AME content of the feed. The results from these analyses indicate that the genetic modification of the corn plant did not influence the metabolism of broilers and laying hens. These results are in agreement with findings published by Aulrich et al. (2001b) and other research groups.

Composition of Eggs and Meat

It can be concluded that plant modifications that increase the resistance of the corn plant towards insects have no significant influence on main components or on the characteristics of the products from the nutritional point of view.

Fate of Corn Chloroplast and Bt Corn Specific DNA

DNA is the basic building block of all life and is present in every cell of all animals, plants, and microbes. Therefore, nearly all food and feedstuffs contain DNA, and animals and man have safely ingested DNA in the past. During consumption of food or feed, normal chemical processes involving gastric acid and miscellaneous enzymes break the macromolecular components like DNA or proteins rapidly into subunits. This effectiveness of digestive degradation in humans and animals is evidenced by the long history of safe consumption of DNA by mammals. All DNA, transgenic or not, consists of the same building blocks and is sensitive to the same digestive processes. An estimation of the intake of recombinant DNA from GM corn leads to the following result: the percentage of recombinant DNA in the genome of *Zea mays* accounts for about 0.00022%. This means that with a content of 1.496 μg of DNA/g of corn, the per capita consumption of recombinant DNA is at a maximum of 0.00033 μg/g (Lassek and Montag, 1990). The highest concentration of intact DNA from the diet can be expected in the crop of birds. In the gizzard, the amount of intact DNA is lower due to degradation through acids and en-

zymes. Even though the DNA turnover in most gut environments is rapid, it is possible that the DNA could be protected against degradation by certain dietary compounds or microenvironments (Schubbert et al., 1994). In human saliva, for example, DNA survives long enough to be capable of transforming a human oral bacterium (Mercer et al., 1999).

The possibility that gene fragments could reach the intestinal epithelia and could then be absorbed into the host organism cannot be eliminated. Model experiments by Doerfler et al. (1997, 1998) showed that phage DNA fragments could still be detected in blood 2 to 8 h, in leukocytes up to 8 h, or in spleen and liver up to 24 h after feeding. Presumably this is the normal method of the body to dispose of such contaminants. It seems possible that foreign DNA fragments could be integrated into cells of the immune system of rodents because they were detectable for several hours within other organs (Doerfler et al., 1997). Doerfler et al. demonstrated the integration of foreign DNA into the mouse genome using fluorescent in situ hybridization or other hybridization techniques. However, long term feeding of mice to 8 generations did not indicate a germline transfer of orally ingested foreign DNA (Hohlweg and Doerfler, 2001). In our study, we analyzed chicken blood, spleen, and liver to clarify the absorption of gene fragments into the host organism. In all chicken tissues such as muscle, liver, and spleen, the short chloroplast gene fragment (*ivr*) was successfully amplified. In contrast, no foreign plant DNA fragments could be found. *Bt* gene specific constructs from the *Bt* corn were not detected in any of the poultry samples (organs, meat, or eggs). That these *Bt*-specific gene fragments could not be found is probably due to their rare occurrence compared with the chloroplast genes. In addition, more than 99.9% of the input DNA was destroyed or degraded to fragments smaller than 180 bp in the intestinal tract, making them beyond the level of detection.

Not all of our samples taken from different birds examined with PCR generated the same results. This means that in some birds, the DNA was more digested than in others and therefore not detectable with PCR. This observation can be attributed to a difference in DNA survival in the intestinal tract of different individual chickens. This is probably due to the difference in the length of transit time between the last releases of food from the crop into the gizzard before they were killed.

Thermal treatment of the feed could affect the detectability of a genetic modification, because it reduces the DNA to shorter fragments. However, if the chosen target sequence is short enough, the degradation of the DNA does not have an impact on the detection.

The suitability of the extraction and analysis procedures were verified through amplification of a chloroplast gene and a poultry-specific fragment. The detection of these fragments indicates that there were no inhibitors and PCR could be performed properly. In addition, the PCR for the *Bt*176-specific fragment showed only strong positive signals for the GM corn and feed samples and no signals for the conventional corn samples. This indicates that cross-contamination of the conventional corn could be excluded.

Polymerase chain reaction results of the eggs were negative. A possible explanation for this observation is that the eggs contain only a small amount of DNA. Therefore, it is very difficult to extract DNA from these samples.

In the future, it is most likely that these small fragments can be detected at very low concentrations with improved methods. So far no research group has found transgenic DNA in milk, meat, or eggs derived from animals receiving GM feed ingredients in their diets. A short chloroplast gene fragment (199 bp) was amplified from all chicken tissues examined, including muscle, liver, spleen, and kidney (Einspanier et al., 2001). In contrast, no transgenic plant DNA fragments were found in eggs or excreta. *Bt* gene specific constructs were not detectable in any of the investigated poultry samples, although Einspanier et al. (2001) used a shorter primer (189 bp). Corn-specific *zein* fragments could be detected in chicken leg muscle, which was ascribed to incomplete degradation of ingested DNA fragments in the gastrointestinal tract of birds (Klotz et al., 2001). One reason for the detection of plant chloroplast genes in chicken samples, in contrast to samples from other animals like cows (Faust and Miller, 1997; Phipps et al., 2001), is that the gastrointestinal tract of chickens, and therefore the digestion path, is shorter. Furthermore, in ruminants, digestion by microorganisms may play an important role in the degradation of DNA.

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